南極に生きる微生物たち

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Microorganisms in Antarctica

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The climate in Antarctica, with its extreme cold, dryness, strong winds, seasonally strong UV radiation and low concentration of organic materials, makes it difficult for most organisms to sustain life. However, a great diversity of microorganisms has been found in icy environments, such as permafrost, polar oceans, snow, lake ice, sea ice, cryoconite holes, and inside of the rocks. Environmental samples were collected by the summer party of the 46th Japanese Antarctic Research Expedition in 2004–2005, and about one thousand of microorganisms are isolated from these samples (Fig. 1). Among them, we introduce the phylogenetically novel microorganisms in this presentation.

I. Novel bacteria isolated from freshwater lakes.

A Gram-stain-negative, non-spore-forming, non-motile, irregularly circular, aerobic/microaerobic appendaged bacterium (strain 120-1^T) was isolated from Naga-Ike, one of the freshwater lakes in the Skarvsnes ice-free area of Antarctica (Fig. 1A, 2A) [1]. Strain 120-1^T grew between 5 and 35°C, with optimum growth at 30°C. Red colonies were formed on plate medium and the carotenoids were present in the cells. Comparative analyses of 16S rRNA gene sequences and physiological characteristics of strain 120-1^T indicate that strain 120-1^T is a phylogenetically novel bacterium, and that it represents a novel species in a new genus, *Rhodoligotrophos* gen. nov., in the order *Rhizobiales*, family *Rhodobiaceae*. The name *Rhodoligotrophos appendicifer* gen. nov. sp. nov. is proposed as the type species of this new genus.

Another rod-shaped, aerobic bacterium (strain 107-E2^T) was isolated from freshwater samples containing microbial mats collected at a lake in Skarvsnes, Antarctica (temporary lake name, Lake Tanago Ike) (Fig. 2B) [2]. Strain 107-E2^T grew between 5 and 25°C, with an optimum of 23°C. Moreover, colony formation was observed on agar media even at -5°C. No growth was observed in media containing organic compounds at high concentrations, which indicated that strain 107-E2^T was an oligotroph. In the late stationary phase, strain 107-E2^T produced a dark brown water-soluble pigment. Esterase, amylase and protease production was observed. Antimicrobial-lytic activities for Gram-negative bacteria and yeast were observed. Analysis of the 16S rRNA gene sequences revealed that strain 107-E2^T belonged to the genus *Lysobacter*, and low DNA–DNA relatedness values with closely related species distinguished strain 107-E2^T from recognized species of the genus *Lysobacter*. The phylogenetic situation and physiological characteristics indicated that strain 107-E2^T should be classified as a representative of a novel species of the genus *Lysobacter*, for which the name *L. oligotrophicus* sp. nov. is proposed.

II. Novel bacteria isolated from white rock collected in the Skallen region.

Interestingly, the interior of the white rock was colorful (green, pink, yellow and brown), suggesting the existence of a cryptoendolithic community containing cyanobacteria (Fig. 1B). When autotrophic bacteria were screened from the crushed rock under light conditions, a green filamentous bacterium (named strain 262-1) covered with several heterotrophic bacteria was obtained. By the cultivation of these bacteria under dark conditions, more than 10 different bacterial species including strains 262-7^T and 262-8^T were obtained [3, 4].

Strain 262-7^T grew at temperatures between 4 and 30°C, with optimal growth at 25°C (Fig. 2C) [3]. Strain 262-7^T showed an unprecedented range of morphological diversity in response to growth conditions. Cells grown in liquid medium were circular or ovoid with smooth surfaces in the lag phase (Fig. 2C$_1$). In the exponential phase, ovoid cells with short projections were observed. Cells in the stationary phase possessed long tentacle-like projections intertwined intricately (Fig. 2C$_2$). By contrast, cells grown on agar plate medium or in liquid media containing organic compounds at low concentration exhibited short- and long-rod-shaped morphology (Fig. 2C$_3$). These projections and morphological variations clearly differ from those of previously described bacteria. According to the phylogenetic position, physiological characteristics and unique morphology variations, strain 262-7^T should be classified as a representative of a novel genus of the family *Sphingomonadaceae*. A novel genus and species with the name *Polymorphobacter multimanifer* gen. nov., sp. nov. was proposed, and the novel species was named after its morphological diversity and formation of unique projections.

Cells of strain 262-8^T are ovoid to rod-shaped and often occur in pairs or chains (Fig. 2D$_1$)[4]. Flagella could be observed by atomic force microscopy (Fig. 2D$_2$). Comparative analyses of 16S rRNA gene sequences and physiological characteristics indicated that strain 262-8^T was a phylogenetically novel bacterium that should be classified in a new genus of the family *Rhodospirillaceae*, for which the name *Constrictibacter antarcticus* gen. nov., sp. nov. is proposed.

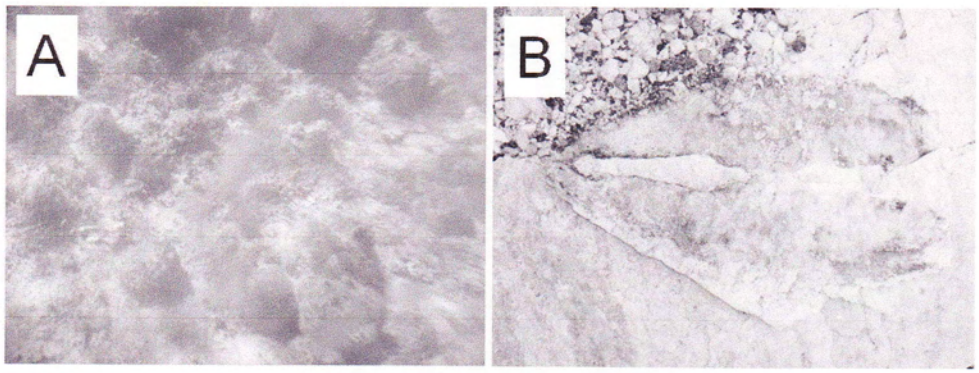

Fig 1. Habitants of microorganisms. (A) Microbial mats constructed on the bottom of freshwater lake in the Skarvsnes region. (B) The white rock collected in the Skallen region.

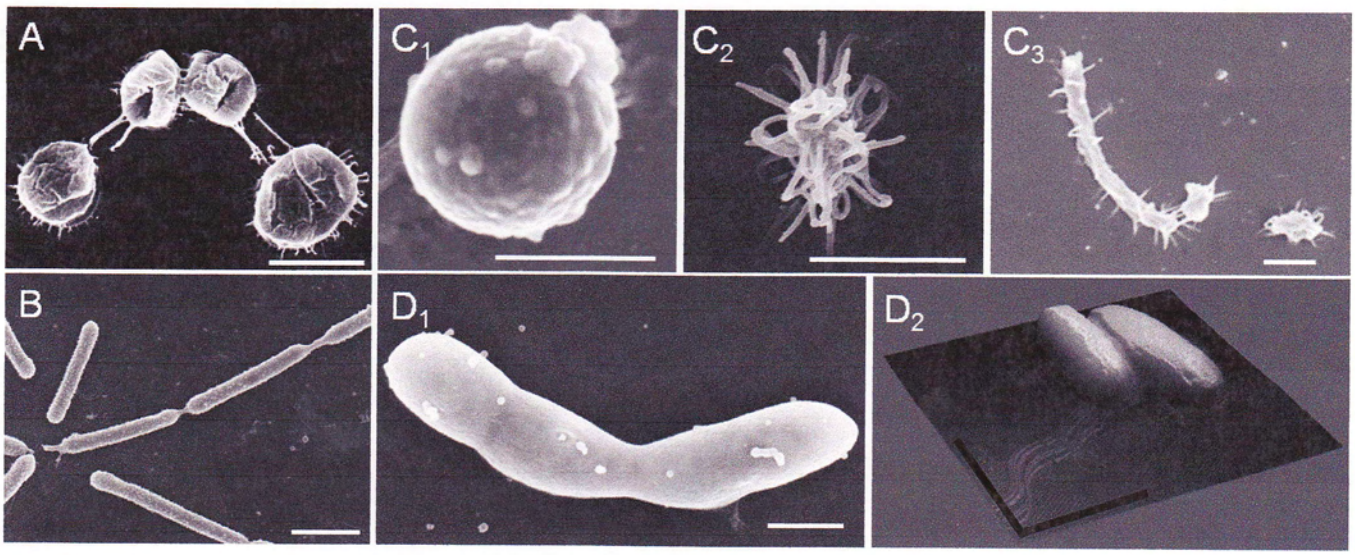

Fig. 2. Micrographs of microorganisms isolated from Antactica. (A) *Rhodoligotrophos appendicifer* 120-1^T. (B) *Lysobacter oligotrophicus* 107-E2^T. (C) *Polymorphobacter multimanifer* 262-7^T; C$_1$, cell in the lag phase; C$_2$, cell in the stationary phase; C$_3$, cells grown on agar plate medium. (D) *Constrictibacter antarcticus* 262-8^T. (A)-(D$_1$), scanning electron micrographs (scale bars, 1 μm); (D$_2$), atomic force micrographs (scale bars, 2 μm).

References

1) Fukuda, W., Yamada, K., Miyoshi, Y., Okuno, H., Atomi, H. and Imanaka, T. *Rhodoligotrophos appendicifer* gen. nov., sp. nov., an appendaged bacterium isolated from a freshwater Antarctic lake. Int. J. Syst. Evol. Microbiol. 62, 194-1950, 2012.

2) Fukuda, W., Kimura, T., Araki, S., Miyoshi, Y., Atomi, H. and Imanaka T. *Lysobacter oligotrophicus* sp. nov., a bacterium isolated from a freshwater lake in Skarvsnes, Antarctica. Int. J. Syst. Evol. Microbiol. 63, 3313-3318, 2013.

3) Fukuda, W., Chino, Y., Araki, S., Kondo, Y., Imanaka, H., Kanai, T., Atomi, H. and Imanaka, T. *Polymorphobacter multimanifer* gen. nov., sp. nov., a polymorphic bacterium isolated from antarctic white rock. Int. J. Syst. Evol. Microbiol. 64, 2034-2040, 2014.

4) Yamada, K., Fukuda, W., Kondo, Y., Miyoshi, Y., Atomi, H. and Imanaka, T. *Constrictibacter antarcticus* gen. nov., sp. nov., a cryptoendolithic micro-organism from Antarctic white rock. Int. J. Syst. Evol. Microbiol. 61, 1973-1980, 2011.